HiTrap IgM Purification, 1 ml

Instructions for Use

HiTrap™ IgM Purification HP is a prepacked, ready to use, column for purification of IgM. The prepacked column, provides fast, simple and easy separations in a convenient format. The column can be operated with a syringe, a peristaltic pump or a liquid chromatography system such as ÄKTA™.

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Important

Please read these instructions carefully before using HiTrap columns.

Intended use

HiTrap columns are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the product in a safe way, please refer to the Safety Data Sheet.

1 Product description

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.

Fig 1. HiTrap, 1 ml column.

Note: *HiTrap columns cannot be opened or refilled.*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiTrap columns.

Column volume (CV)	1 ml
Column dimensions	0.7 × 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/ 16"	For sealing bottom of HiTrap column	2, 5 or 7

Chromatography medium properties

IgM Purification HP is a thiophilic affinity medium with 2-mercaptopyridine coupled to Sepharose™ High Performance. Thiophilic adsorption was first described by Porath et al. (Ref. 1) and is promoted by water-structuring salts. The interaction between protein and ligand has been suggested to result from a combined electron donating and accepting action of the ligand (Ref. 2) or alternatively as a mixed mode hydrophilichydrophobic interaction (Ref. 3). The base matrix is a rigid, highly crosslinked, beaded agarose with high chemical stability.

The main application area for HiTrap IgM Purification HP is purification of IgM, but it can also be used for purification of other immunoglobulins.

The characteristics of the medium are summarized in table 2.

Table 2. IgM Purification HP characteristics

Ligand	2-mercaptopyridine
Ligand concentration	2 mg/ml
Binding capacity[1]	5 mg human IgM/ml medium
Mean particle size	34 µm
Bead structure	Highly cross-linked spherical agarose
Maximum flow rate	4 ml/min
Recommended flow rate	0.1–1 ml/min
pH stability[2]	
Long term	3 to 11
Short term	2 to 13
Temperature stability	
Regular use	4°C to room temperature
Storage	2°C to 8°C
Storage buffer	20% ethanol

[1] Running conditions according to the recommendations in this instructions.

[2] **pH stability, long term** refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance. **pH stability, short term** refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.

2 Operation

Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use.

Binding 20 mM sodium phosphate, 0.8 M $(NH_4)_2SO_4$, pH 7.5

Elution buffer: 20 mM sodium phosphate, pH 7.5

Regeneration: 20 mM sodium phosphate, pH 7.5 with 30% isopropanol

Sample preparation

The sample must have the same concentration of ammonium sulphate as the binding buffer. Perform buffer exchange using HiTrap Desalting, HiPrep™ 26/10 Desalting, or PD-10 Desalting columns or gradually add small amounts of solid ammonium sulphate to the sample until the final concentration is, for example, 0.8 M. Stir slowly and continuously during this procedure. Pass the sample through a 0.45 µm filter immediately before loading on the column.

Note: *It is important to add the ammonium sulphate gradually to avoid precipitation of IgM.*

Purification

The recommended flow rate for HiTrap IgM Purification HP is 1 ml/min.

1. Fill the syringe or pump tubing with buffer. Remove the stopper and connect the column to the syringe (with the provided luer connector), or pump tubing, "drop to drop" to avoid introducing air into the column.
2. Remove the snap-off end at the column outlet.
3. Wash the column with 5 column volumes of each buffer: Binding buffer, elution buffer and regeneration buffer.
4. Equilibrate the column with 5 column volumes of binding buffer.
5. Apply the sample using a syringe fitted to the luer connector, or by pumping it onto the column.
6. Wash out unbound sample with 15 column volumes of binding buffer or until no material appears in the effluent.
7. Elute the IgM with 12 column volumes of elution buffer.
8. Regenerate the column with 7 column volumes of regeneration buffer.

9 Re-equilibrate the column with 5 column volumes of binding buffer.

Note: *The reuse of HiTrap IgM Purification HP depends on the nature of the sample and should only be performed with identical samples to prevent cross-contamination.*

Note: *If a P-1 pump is used a max flow rate of 1-3 ml/min can be run on a HiTrap 1 ml column packed with Sepharose High Performance media.*

Binding

Not all monoclonal IgM may bind to the HiTrap IgM Purification HP column at 0.8 M ammonium sulphate. Binding can be improved by increasing the ammonium sulphate concentration to 1.0 M. However, an increased concentration of ammonium sulphate will cause more IgG to bind, which might be a problem if the sample is serum or if serum has been added to the cell culture medium. If the purified IgM is contaminated by IgG, the IgG can be removed by using for example HiTrap rProtein A FF. Alternatively, the ammonium sulphate can be exchanged for 0.5 M potassium sulphate. Most monoclonal IgM bind to the column in the presence of 0.5 M potassium sulphate and the purity of IgM is comparable to the purity achieved with 0.8 M ammonium sulphate.

Elution

Some monoclonal IgM may bind too tightly to HiTrap IgM Purification HP for total elution with elution buffer. The remaining IgM will be eluted with the regeneration buffer, but the high content of isopropanol will cause precipitation of IgM and immediate buffer exchange, using HiTrap Desalting, PD-10 Desalting column or dilution of the sample is required to preserve the IgM. Lower concentrations of isopropanol may elute the IgM and decrease the risk of precipitation.

3 Scaling up

For quick scale-up of purifications, two or three HiTrap IgM Purification HP columns can be connected in series (backpressure will be higher).

4 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Fig 2. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 2) may damage the column.*

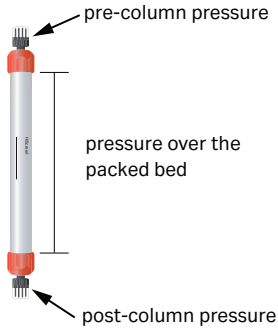

Fig 2. Pre-column and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

1. Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2. Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3. Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value.

   The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Note: *Repeat the procedure each time the parameters are changed.*

5 Storage

Store the column at 2°C to 8°C in 20% ethanol.

6 Ordering information

Product	No. Supplied	Code No.
HiTrap IgM Purification HP	5 × 1 ml	17-5110-01

Related products	No. Supplied	Code No.
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
PD-10 Desalting Column	30	17-0851-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column*)	2	18-1112-51
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column*)	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16" *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Related literature	Code No.
Antibody Purification Handbook, Principles and Methods	18-1037-46
Solutions for Antibody Purifications, Selection Guide	28-9351-97
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media, Selection Guide	18-1121-86

7 References

1. Thiophilic adsorption – a new method for protein fractionation. FEBS Lett., 1985, 185, 306-310. Porath J, Maisano F and Belew M.
2. Thiophilic adsorption: a comparison of model protein behavior. Biochemistry, 1987, 26, 7199-7204. Hutchens TW and Porath J.
3. Investigations on the specificity of thiophilic interaction for monoclonal antibodies of different subclasses. J. Chrom. B, 1996, 675, 197-204. Finger UB, Brümmer W, Knieps E, Thömmes J and Kula M-R.

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